

## Research

### Corresponding author

Shaw Watanabe, MD, PhD

Lifescience Promoting Association

Daikyo-cho 25-3-1004

Shinjuku-ku, Tokyo 160-0015

Japan

Fax: +81-3-5379-7786

E-mail: [watashaw@lifescience.or.jp](mailto:watashaw@lifescience.or.jp)

Volume 3 : Issue 1

Article Ref. #: 1000DROJ3129

### Article History

Received: December 30<sup>th</sup>, 2016

Accepted: January 24<sup>th</sup>, 2017

Published: January 25<sup>th</sup>, 2017

### Citation

Watanabe S, Hirakawa A, Utada I, et al. Ketone body production and excretion during wellness fasting. *Diabetes Res Open J.* 2017; 3(1): 1-8. doi: [10.17140/DROJ-3-129](https://doi.org/10.17140/DROJ-3-129)

### Copyright

©2017 Watanabe S. This is an open access article distributed under the Creative Commons Attribution 4.0 International License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

# Ketone Body Production and Excretion During Wellness Fasting

Shaw Watanabe, MD, PhD<sup>1\*</sup>; Azusa Hirakawa, BS<sup>1,2</sup>; Izumi Utada, BS<sup>3</sup>; Seiichiro Aoe, PhD<sup>2</sup>; Satsuki Moriyama, MS<sup>4</sup>; Satoko Hitomi, BS<sup>3</sup>; Hiroko Honda, BS<sup>3</sup>; Reiko Takiguchi, BS<sup>3,4</sup>; Ryota Haba, PhD<sup>1</sup>; Tanji Hoshi, MD, PhD<sup>1,5</sup>

<sup>1</sup>Lifescience Promoting Association, Daikyo-cho 25-3-1004, Shinjuku-ku, Tokyo 160-0015, Japan

<sup>2</sup>Otsu Women's University, Sanbancho 12, Chiyoda, Tokyo 102-8357, Japan

<sup>3</sup>AOB Keioh Group CORP., Kobuchizawa-cho 2961, Hokuto City, Yamanashi 408-8522, Japan

<sup>4</sup>ARSOA HONSHA CORP., Shibuya 3-26-8, Shibuya-ku, Tokyo 150-0002, Japan

<sup>5</sup>Tokyo Metropolitan University, Minami-osawa 1-1, Hachioji City, Tokyo 192-0397, Japan

## ABSTRACT

**Introduction:** The importance of ketone body metabolism in diabetic patients has been known since the 1970s, but recently  $\beta$ -hydroxybutyrate ( $\beta$ HB) has been identified as a key component of a metabolic signaling pathway. However, many questions remain unanswered about individual differences in the production and excretion of ketone bodies.

**Objectives:** Fourteen healthy subjects were admitted in the ARSOA Wellness Fasting program. During a 4-day fasting period, they received a combination of very low-energy vegetarian diet, physical exercise, meditation, and lectures about healthy lifestyles.

**Methods:** Every morning, participants measured their body composition, blood glucose, and  $\beta$ -hydroxybutyrate ( $\beta$ HB). The first urine of the day was analyzed for  $\beta$ HB, glucose, pH and 7 other elements. Ten milliliters of urine were deep frozen for further quantitative analysis. Peripheral blood was collected on days 1 and 4 for routine biochemical analyses and for the determination of serum levels of hormones. All data were collated in an Microsoft Excel<sup>®</sup> file, and transferred to SPSS for statistical analysis.

**Results:** Ketone bodies in the blood increased from  $0.3 \pm 0.2$  mM at day 1 to  $1.8 \pm 0.9$  mM at day 4 of fasting, while the glucose level decreased from  $5.8 \pm 1.9$  mM to  $4.5 \pm 1.5$  mM. Increases in peripheral blood  $\beta$ HB were assigned to 3 groups (0.8-1.2 mM, 1.3-1.8 mM, 1.8-4.8 mM). Pass analysis suggested that decreased insulin and glucose levels under 4.5 mM both seemed to trigger ketogenesis. The  $\beta$ HB/Acetoacetate (AcAc) ratio in blood was about 5, regardless of the  $\beta$ HB concentration, but the range varied from 0.56 to 3.36 in the urine. The highest excreted concentration of  $\beta$ HB was 597 mmol/g creatinine. These values returned to normal ranges on the 14<sup>th</sup> day (10 days after fasting). Changes in blood ketone bodies significantly correlated with total thyroxin, while urinary epinephrine changes correlated with urinary ketone bodies. Ketone body production seemed to cause hepatic and renal damage, as indicated by a slight alteration in markers of hepatic function and a 12% decrease in eGFR.

**Conclusions:** Four days of fasting induces hyperketosis with some metabolic changes. The magnitude of changes in ketone body concentrations correlates with the levels of insulin, glucose, triacylglycerol and several hormones. The urinary excretion of  $\beta$ HB abruptly increased in group 3 (more than 1.8 mM). It was 100 times higher than the blood  $\beta$ HB concentration. Further studies are necessary to establish the safety of fasting as a way to induce hyperketosis for therapeutic needs. Our observations of a slight decrease in hepatic function and a 12% decrease in eGFR call for caution.

**KEYWORDS:** Fasting; ketone bodies; Acetoacetate;  $\beta$ -hydroxybutyrate; Glucose; Energy metabolism; Hormones; eGFR; Intervention study.

**ABHREVIATION:** AcAc: Acetoacetate;  $\beta$ HB:  $\beta$ -hydroxybutyrate; eGFR: Estimated Glomerular Filtration Rate.

**INTRODUCTION**

Ketoacidosis is sometimes a fatal complication of diabetes mellitus. Ketone bodies are typically produced in diabetic patients, but also by ketogenic diets and by fasting.

In the 1970's the kinetics of ketone bodies were extensively studied after fasting and exercise.<sup>1-6</sup> In the 1980's various effects of hormones and fatty acids were reported.<sup>7-18</sup> In the 1990's  $\beta$ HB was found to be more than just a metabolite, having important cellular signaling roles.<sup>19-23</sup> Notably, the inhibitory action on histone-demethylase opened prospects for therapeutic applications for various diseases. Recently ketone-ester has been recommended as a dietary supplement for athletes.<sup>23</sup>

We have previously proposed the "dual engine" hypothesis to explain shifts in energy production: Basic ketone engine and booster glucose engine.<sup>24,25</sup> However, individual differences in ketone production and the dynamics of ketogenesis are still unclear. Therefore, we tried to clarify the occurrence of ketogenesis during fasting by measuring concurrent changes in glucose, ketone bodies, fatty metabolism and various hormonal changes in both blood and urine. We recruited study subjects among participants in a comprehensive fasting program, which included a balanced diet, physical activity, and mind exercises to achieve a healthy spiritual life.

**METHODS**

**Subjects**

Fourteen healthy subjects admitted in the ARSOA Wellness Fasting program participated in the study between 10-13 September 2016.<sup>26</sup> All participants gathered at the Relache Holistic Center in *Megami-no-mori*, Hokuto City, Yamanashi prefecture. Persons who wished to participate in the program were telephone-interviewed by an expert staff about their health

and their willingness to join the program. Healthy adults of any age or gender could to participate in the program. We excluded pregnant women and patients receiving any medication for cardiovascular disease, diabetes, chronic infectious diseases, gastro-intestinal tract conditions, or cancer.

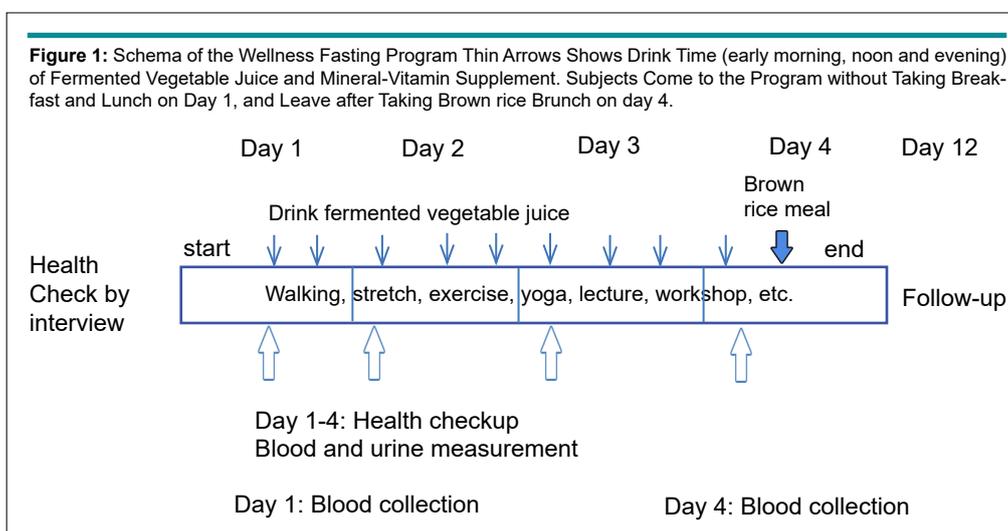
All participants signed a consent sheet after having received precise explanations about of the program. The program was approved by the Ethical Committee in the Life Science Promotion Association.

**Program**

The schedule and contents of wellness fasting program has been previously reported.<sup>26</sup> During the 4-days fasting program, participants received a combination of very low-energy vegetarian diet (about 300 kcal per day intake from fermented vegetable juice), physical exercise (2-hours walking, slow training, and stretching), meditation, and lectures about healthy lifestyles (Figure 1).

Every morning participants measured their body composition using the Body Composition Analyzer<sup>®</sup> (Tanita, Tokyo, Japan), which determines body weight, fat volume, muscle volume, water contents, estimated bone weight, basal metabolic rate and body mass index (BMI), based upon the electric impedance.<sup>27</sup> Blood pressure and pulse rate were measured with the Omron HEM-7021<sup>®</sup> monitor (Kyoto, Japan).

Blood glucose and  $\beta$ -hydroxybutyrate ( $\beta$ HB) were measured every morning with the Freestyle Precision Neo<sup>®</sup> meter (Abbott, Chicago, IL, USA).<sup>28</sup> Daily first urine was checked for urinary pH, glucose,  $\beta$ HB, urobilinogen, proteins, occult blood, bilirubin, nitrate, and relative gravity using the Uropaper III<sup>®</sup> (Eiken, Tokyo, Japan). A sample of about 10 ml of urine was stored in a frozen tube for quantitative analysis of creatinine, acetoacetate (AcAc),  $\beta$ HB and catecholamines by the Serum Research Laboratory (SRL).<sup>29</sup>



All participants were requested to send urine at the 10<sup>th</sup> day after the fasting.

### Laboratory Analyses

Sera at days 1 and 4 were quantitatively analyzed for ketone bodies (acetone, AcAc,  $\beta$ HB), insulin, glucagon, growth hormone, triiodo-thyronin, total thyroxin, triacyl-glycerol, free fatty acids, AST, ALT,  $\gamma$ GTP and creatinine. The estimated glomerular filtration rate (eGFR) was calculated from age and creatinine value using the formula [male; eGFR (ml/min/1.73 m<sup>2</sup>)=194 $\times$ [age]<sup>-0.287</sup> $\times$ [CRE]<sup>-1.094</sup>, females; eGFR (ml/min/1.73 m<sup>2</sup>)=male\_eGFR 0.739] recommended by the Japanese Renal Society.<sup>30</sup>

All urinary samples were quantitatively analyzed for creatinine, ketone bodies and catecholamines (epinephrine, norepinephrine and dopamine). Urine concentrations of these chemicals were standardized relative to creatinine levels.

### Statistical Analysis

All data were reported in an Microsoft Excel<sup>®</sup> spreadsheet, and transferred to SPSS Version 22 (IBM SPSS, Tokyo, Japan). After double-checking all entries, aggregate data were expressed by mean and standard deviation (SD) if the distribution was normal. Otherwise, median and range were calculated, and non-parametric tests were used. The level of statistical significance was set at  $p < 0.05$ . We also used Pass analysis to examine presumptive underlying variables by AMOS Version 22 for Windows (IBM Inc., New York, USA).<sup>31</sup> The estimation of the best fitting model was carried out by the method of maximum likelihood of Pass analysis. Results were regarded as good model, if the RMSEA was less than 0.05.

## RESULTS

The anthropometric data of subjects, basal metabolic rate (BMR)

and body mass index (BMI) are shown in Table 1. Participants were 12 women and 2 men, ranging in age from 28 to 75. Sex differences in biochemical data were not apparent, so both genders were combined for statistical analysis. The magnitude of body weight loss after fasting was 2 kg on average. The rate of body fat consumption was variable, but visceral fat decreased especially among group 3 participants, if subjects were categorized by  $\beta$ HB concentration, as shown later. The systolic blood pressure decreased by 12 mmHg on average, and notably by 15 mmHg in diastolic pressure in group 3. On the contrary, the pulse rate increased. The body temperature decreased by 0.2-0.4 °C. Subjective symptoms, such as headache, hunger, cold feeling, and gastro-intestinal distress were improved toward the last day of fasting.

Ketone bodies in the blood increased from  $0.3 \pm 0.2$  mM at day 1 to  $1.8 \pm 0.9$  mM at day 4 of fasting, while glucose levels decreased from  $5.8 \pm 1.9$  mM to  $4.5 \pm 1.5$  mM on average (Figure 2). On day 4, in parallel with the increase in blood ketone bodies, the urinary excretion of AcAc increased to 40.9 mM (7.76-159.2),  $\beta$ HB 25.1 mM (2.72-597.0) and total ketone bodies 66.1 mM (10.5-756.5) (Table 2). These deviations returned to 0.16 mM (0-10.1) after 14 days (10 days after fasting).

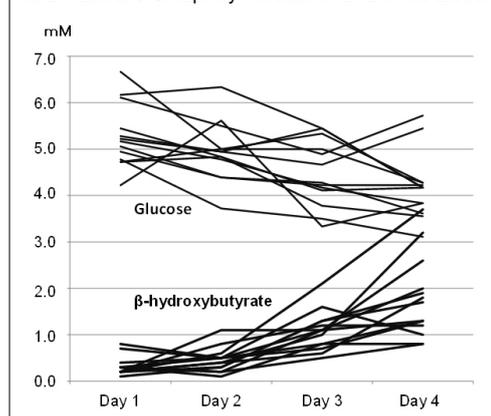
The increase in  $\beta$ HB could be divided in 3 groups depending on the blood  $\beta$ HB level at the 4<sup>th</sup> day (0.8-1.2mM, 1.3-1.8 mM, 1.9-4.8 mM) (Table 3). The three groups showed different patterns in the production and urinary excretion of ketone bodies (Figure 3). The blood  $\beta$ HB/AcAc ratio was similar between the 3 groups, but the urine ratio was 0.56 in group 1, 0.75 in group 2 and 3.36 in group 3. The maximal excretion of  $\beta$ HB in the urine was 597.9 mmol/g creatinine. It was 100 times more of the blood  $\beta$ HB concentration.

Decreases in insulin and inverse glucagon increases showed dose-dependent changes by groups. Decreased triacylglycerol and increased free fatty acids were largest in group 3. High ketosis in groups 2 and 3 were associated with

**Table 1:** Anthropometric Data of Participants to 4-Day Wellness Fasting.

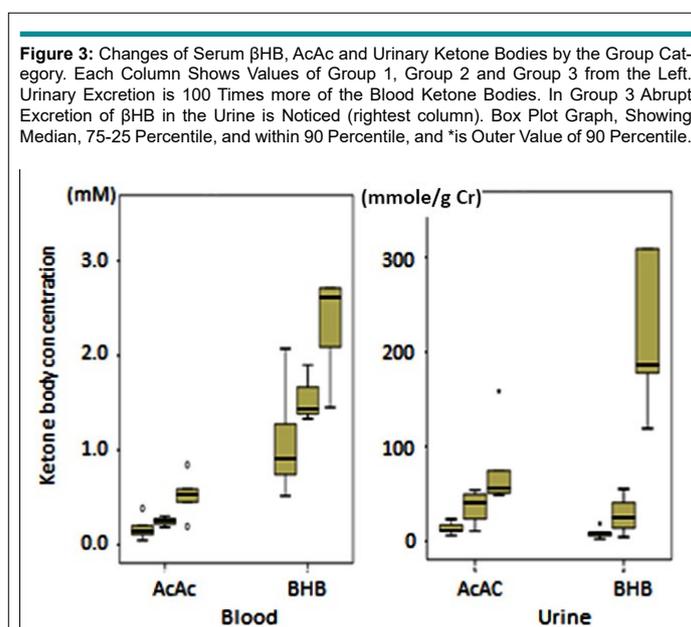
Age (year)	51.0 $\pm$ 14.4
Height (cm)	158.0 $\pm$ 7.4
Weight (kg)	54.9 $\pm$ 10.7
Body fat percent (%)	29.7 $\pm$ 8.2
Water percent (%)	49.5 $\pm$ 4.4
Muscle_volume (kg)	36.2 $\pm$ 5.8
Water_weight (kg)	27.0 $\pm$ 4.9
Bone_weight (kg)	2.1 $\pm$ 0.4
BMR (kcal/day)	1119 $\pm$ 169
BMI (kg/m <sup>2</sup> )	22.0 $\pm$ 3.9

**Figure 2:** Individual Changes of Blood Glucose and  $\beta$ -hydroxybutyrate during 4-Day Fasting Subjects could be Divided to 3 Groups by Increase of BHB in the Blood.



	Day 1	Day 2	Day 3	Day 4	Day 14
Blood glucose (mM)	5.8±1.9	5.1±0.7	4.8±1.3	4.5±1.5	
Blood βHB (mM) <sup>1</sup>	0.3±0.2	0.5±0.3	1.1±0.4	1.8±0.9	
Blood βHB (mM) <sup>2</sup>	0.17±0.17			2.0±1.0	
Blood AcAc	0.1±0.05			0.4±0.2	
Urinary pH	5.9±1.3	5.4±0.5	5.5±0.5	5.4±0.5	
Urinary glucose	-	-	-	-	-
Urine AcAc (mM)	0.1(0-29.8)	1.2 (0.1-16.4)	18.1 (2.0-46.3)	40.9 (7.76-159.2)	0.1 (0.02-6.2)
Urine βHB (mM)	0.1(0-15.1)	0.6 (0.1-10.9)	8.0 (1.0-116.2)	25.1 (2.72-597.0)	0.1 (0.06-3.85)
Total Ketone bodies (mM)	0.2 (0-44.9)	1.0 (0.1-26.2)	29.7 (3.2-192.1)	66.1 (10.5-756.5)	0.16 (0-10.1)

Median (min-max) urinary ketone body concentration is standardized by urinary creatinine (g)  
<sup>1</sup>Abbott precision; <sup>2</sup>Quantitative method



low glucose level less than 4.5 mM.

Growth hormone, tri-iodothyronin, and total thyroxin did not show significant change by groups, although total thyroxin (T4) correlated with blood ketone bodies, and urinary epinephrine significantly correlated with urinary ketone bodies (Table 3). AST and ALT slightly increased, while γGTP decreased in group 3.

Creatinine was slightly increased and the estimated glomerular filtration rate (eGFR) decreased by about 13% in all groups.

Pass analysis could resolve a complex relationship between the above described factors and the production of ketone bodies (Figure 4). Changes in insulin levels seemed to be the most effective factor to initiate an increase in βHB, followed

by changes of BMR and body weight.

## DISCUSSION

It has been shown that a few days of fasting causes many metabolic changes, involving various hormones and free fatty acid.<sup>32</sup> Takahira et al<sup>33</sup> was at the vanguard of fasting therapy in 1910-1920. He studied precisely the metabolic changes caused by fasting, and reviewed the efficacy of fasting therapy in various disease states. Mitsuo Koda<sup>34</sup> developed fasting dietary therapy for various diseases, and confirmed beneficial effects for many patients with intractable diseases.

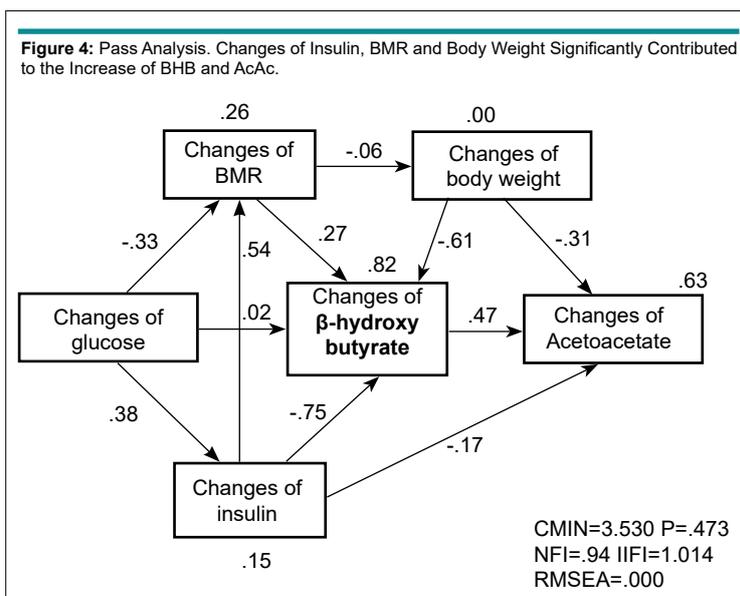
Cahill et al<sup>1</sup> studied metabolic changes during 40 days of starvation and found that β-hydroxybutyrate replaced glucose as a source of energy. Fasting causes many metabolic changes,<sup>1,5,6,8</sup> including a switch in cell fuel from glucose to fatty

**Table 3:** Pearson's Correlation Coefficient between Selected Factors (Changes of Concentrations of Each Factors).

Pearson's correlation coefficients between various changes of factors during fasting																
	AcAc	βHB	u_AcAc	u_βHB	Age	BMR	temp	sbp	dbp	TG	FFA	Creation	eGFR	AST	ALT	γ GTP
AcAc	1	.971**	.630*	.706**	-0.249	0.096	0.400	-0.058	-0.422	0.474	0.092	-0.207	0.135	-0.162	-0.263	-0.068
βHB	.971**	1	0.537	.622*	-0.347	0.120	0.382	0.001	-0.369	0.391	0.115	-0.151	0.069	-0.165	-0.250	-0.047
u_AcAc	.630*	0.537	1	.952**	-0.043	-0.045	.536*	-0.276	-0.593*	.701**	0.041	0.218	-0.200	-0.331	-0.467	0.332
u_βHB	.706**	.622*	.952**	1	-0.009	-0.007	0.445	-0.239	-.562*	.615*	-0.160	0.155	-0.228	-0.331	-0.467	0.332
Insulin	-0.321	-0.197	-.655*	-0.522	0.149	0.421	-0.172	0.388	0.398	-0.445	-0.165	-0.022	-0.139	0.480	0.527	0.104
Glucagon	-0.371	-0.291	-0.035	-0.097	-0.357	-0.348	-0.274	0.176	0.152	-0.098	-0.067	.588*	-0.537	-0.415	-0.249	0.141
Growth hor	-0.007	-0.011	-0.286	-0.249	-0.213	-0.398	-0.379	0.075	0.516	-0.426	0.390	-0.493	0.455	0.344	0.246	-.6723*
T3	0.135	0.093	0.205	0.136	0.451	.678*	.750**	-0.380	-0.472	0.344	0.039	-0.069	0.092	0.065	-0.008	0.301
T4	.603*	.679*	0.289	0.433	-0.317	0.217	0.181	0.228	-0.132	0.197	-0.178	0.364	-0.445	-0.031	0.046	0.134
Epinephrine	0.515	0.442	.747**	.681**	0.229	0.097	.579*	-0.159	-0.407	.636*	0.114	-0.136	0.66	-0.123	-0.204	0.308
Norepi	0.386	0.339	0.124	0.255	-0.025	-0.425	-0.362	-0.199	-0.123	-0.234	-0.047	-0.492	0.454	-0.301	-.635*	-0.434
Dopamin	0.235	0.181	-0.092	-0.030	0.003	-0.318	-0.273	-0.960	-0.099	-0.163	0.017	-0.501	0.476	-0.326	-0.522	-.717**

\*\*p<0.01, \*p<0.05

T3: Triiodothyronin; T4: Total thyroxin, norepi: norepinephrine; BMR: Basic metabolic rate; temp: Body temperature; sbp: systolic blood pressure; dop: diastolic blood pressure; TG: triacylglycerol; FFA: free fatty acids; eGFR: estimated Glomerular Filtration Rate.



acids,<sup>24</sup> but excretion of ketone bodies into the urine would cause energy loss.

The hyperglycemia and hyperketonemia of diabetic ketoacidosis are primarily initiated by the overproduction of ketone bodies, such as acetone, AcAc and βHB. Felty and Balasse<sup>13</sup> demonstrated the production and disposal of ketone bodies in diabetic ketosis by using <sup>14</sup>C-β-hydroxybutyrate or <sup>14</sup>C-acetoacetate constant infusions in 13 insulin-dependent type-1 diabetic patients and 26 control subjects fasted for 15 hours to 23 days. Both groups showed a similar range of ketone body levels (1-12 mM) and turnover. The use of <sup>14</sup>C isotopic methods is no longer possible now-a-days for ethical reasons, so their data should be considered the gold standard of ketone body turnover. They showed that ketone body production (a few

mmol/min/1.73 m<sup>2</sup>) was correlated with plasma ketone body concentration, which reached a plateau after 3 days of fasting, and it was modified by the rate of metabolic disposal.

In their control group, ketonemia varied between 0.09 and 11.9 mM, and 22 subjects had total KB levels exceeding 2 mM with an average βHB/AcAc ratio 3.72±0.18. This group was similar to our group 3 subjects. They showed a curvilinear relationship between the metabolic clearance rate of total ketones and plasma ketone body concentration. A maximal metabolic disposal rate of about 2.3 mmole/min/1.73 m<sup>2</sup> was attained in subjects for ketone concentrations exceeding 9 mM, corresponding to 331 mmole/day. The exponential pattern suggested that the removal capacities of tissues, such as muscle and brain, are progressively saturated as concentration rises. The

hyperketonemia of fasting is primarily caused by an increased production of ketones, but the phenomenon is amplified by a progressive limitation in the ability of tissues to extract ketones from blood.

Keller et al<sup>17</sup> using <sup>14</sup>C-AcAc and Miles et al<sup>12</sup> using <sup>14</sup>C-βHB reported similar ketone production rates (about 1.2 mmole/min) in type-1 diabetic patients several hours after the interruption of an insulin infusion, but there was a more than threefold difference in the concentration of ketones, which averaged 2.2 mM in the Keller's study and 7 mM in Miles' study. It may be influenced by a faster removal of AcAc from blood to the tissue. Interestingly glucose homeostasis was very stable. Reichard et al<sup>20</sup> estimated the average plasma glucose as 2.1 mM come from plasma acetone, and the proportion of 1,2-propanediol derived from plasma acetone as 74%. This should be needed for further confirmation.

Kellers et al<sup>11</sup> reviewed the roles of insulin, catecholamines, and thyroid hormones in the regulation of ketone body kinetics. Insulin corrected diabetic ketoacidosis (DKA) largely *via* the suppression of lipolysis. In our study, inverse changes in insulin and glucagon were noteworthy. In addition, decreased triacylglycerol and increased free fatty acids appeared to be consumed for the production of ketone bodies by β-oxidation of fatty acids. Glucagon was said to be important in determining the magnitude of ketone body production for a given degree of FFA availability and insulin deficiency.<sup>10</sup>

On the contrary, the elevation of plasma glucagon during Intralipid® infusion failed to affect ketogenesis, and the βHB/AcA concentration ratio decreased significantly ( $p < 0.01$ ).

In case of diabetic ketoacidosis, insulin administration is a effective therapy. Keller's data indicate that the elevation of plasma insulin to high physiological concentrations restrains FFA-induced ketogenesis.<sup>16,17</sup> Insulin administration lowers ketone body concentrations by three independent mechanisms: First, it inhibits lipolysis, and thus lowers free fatty acid availability for ketogenesis; second, it restrains ketone body production within the liver; third, it enhances the utilization of peripheral ketone body.<sup>19</sup>

As for the hormonal effects, thyroid hormones are said to regulate lipolysis, intrahepatic ketogenesis, and peripheral ketone body utilization,<sup>17</sup> Norepinephrine has also been reported to increase ketogenesis, not only by stimulating lipolysis and releasing free fatty acids, but also by increasing intra-hepatic ketogenesis.<sup>18</sup> In our study, changes of total thyroxin significantly correlated with blood ketone bodies, while changes of urinary epinephrine correlated with urinary ketone bodies, and dopamine showed significant decrease in group 3. Insufficient statistical power in our study could not clarify further effects of hormones, but the Pass analysis could suggest that decreased BMR and insulin significantly contributed to the increase in βHB and AcAc. A further larger study could clarify the above hormonal

effects and kinetics of ketone bodies by a flux analysis.

Balasse et al<sup>13</sup> observed that the blood concentrations, production rate and urinary elimination of total ketones after 3 days of fasting were  $7.09 \pm 0.32$  mM,  $1.91 \pm 0.08$  mmole/min, and  $0.167 \pm 0.014$  mmole/min, respectively. Urinary excretion was 24 mmol/day. These values correspond to our findings in group 2. The very high excretion rate of βHB in the urine in group 3 suggested that all produced βHB was excreted in the urine. This may cause renal damage as observed by a 13-14% decrease in eGFR in our study. Ketone bodies inhibit aminoacid metabolism and protein turnover in skeletal muscles, which may be an important survival mechanism during adaptation to catabolic states such as prolonged fasting.<sup>2,6</sup>

Risks and benefits of βHB should be further clarified, because slight liver and renal damages were suggested by increased AST, ALT, and a 12% decrease in eGFR.

Limitations of the present study is a small number of subjects that results in weak statistical power. We are now expanding the number of participants, and obtained nearly 60 subjects under the same protocol. We also try to analyze intestinal microbiota before and after fasting whether or not they contribute to the ketone body metabolism. The intention of this paper is to call attention about the efficacy and safety of fasting for raising ketone bodies for wellness and/or therapeutic aids.

## CONCLUSION

Four days of fasting induces hyperketosis with some metabolic changes. The magnitude of changes in ketone body concentrations correlates with the levels of insulin, glucose, triacylglycerol and several hormones. The urinary excretion of βHB abruptly increased in group 3 (more than 1.8 mM). It was 100 times higher than the blood βHB concentration.

Further studies are necessary to establish the safety of fasting as a way to induce hyperketosis for therapeutic needs. Our observations of a slight decrease in hepatic function and a 12% decrease in eGFR call for caution.

## ACKNOWLEDGEMENT

The authors thank participants and the staff of the wellness fasting program for their cooperation. We appreciate Dr. Philippe Calain for his constructive discussion and revising this manuscript.

## CONFLICTS OF INTEREST

The study was supported by the ARSOA Educational Program.

## REFERENCES

1. Cahill GF Jr. Starvation in man. *New Engl J Med.* 1970; 282:

668-675. doi: [10.1056/NEJM197003192821209](https://doi.org/10.1056/NEJM197003192821209)

2. Owen OE, Reichard GA Jr. Human forearm metabolism during progressive starvation. *J Clin Invest.* 1971; 50(50): 1536-1545. Web site. <http://www.jci.org/articles/view/106639>. Accessed December 29, 2016.

3. Owen OE, Markus H, Sarshik S, Mozzoli M. Relationship between plasma and muscle concentrations of ketone bodies and free fatty acids in fed, starved and alloxan-diabetic states. *Biochem J.* 1973; 134(2): 499-506. doi: [10.1042/bj1340499](https://doi.org/10.1042/bj1340499)

4. Blasse EO. Changes induced by exercise in rates of turnover and oxidation of ketone bodies in fasting man. *J Appl Physiol.* 1978; 44(1): 5-11. Web site. <http://jap.physiology.org/content/44/1/5.long>. Accessed December 29, 2016.

5. Blasse EO. Kinetics of ketone body metabolism in fasting humans. *Maetabolism.* 1979; 28(1): 41-50. doi: [10.1016/0026-0495\(79\)90166-5](https://doi.org/10.1016/0026-0495(79)90166-5)

6. Fely F, Balasse EO. Ketone body turnover during and after exercise in overnight-fasted and starved humans. *Am J Physiol.* 1983; 245: E318-E325. Web site. <http://ajpendo.physiology.org/content/245/4/E318>. Accessed December 29, 2016.

7. Thompson JR, Wu G. The effect of ketone bodies on nitrogen metabolism in skeletal muscle. *Comp Biochem Physiol B.* 1991; 100(2): 209-216. doi: [10.1016/0305-0491\(91\)90363-I](https://doi.org/10.1016/0305-0491(91)90363-I)

8. Robinson AM, Williamson DH. Physiological role of ketone bodies as substrates and signals in mammalian tissues. *Physiol Rev.* 1980; 60(1): 143-187. Web site. <http://physrev.physiology.org/content/60/1/143.long>. Accessed December 29, 2016.

9. Schade DS, Eaton RP. Metabolic and clinical significance of ketosis. *Spec Top Endocrinol Metab.* 1982; 4: 1-27.

10. Miles JM, Haymond MW, Gerich JE. Effects of free fatty acids, insulin, glucagon and adrenaline on ketone body production in humans. *Ciba Found Symp.* 1982; 87: 192-213. doi: [10.1002/9780470720691.ch11](https://doi.org/10.1002/9780470720691.ch11)

11. Keller U, Gerber PP, Stauffacher W. Stimulatory effect of norepinephrine on ketogenesis in normal and insulin-deficient humans. *Am J Physiol.* 1984; 247(6 Pt 1): E732-E739. Web site. <http://ajpendo.physiology.org/content/247/6/E732>. Accessed December 29, 2016.

12. Miles JM, Gerich JE. Glucose and ketone body kinetics in diabetic ketoacidosis. *Clin Endocrinol Metab.* 1983; 12(2): 303-319.

13. Felty R, Balasse EO. Ketone body production and disposal in diabetic ketosis. A comparison with fasting ketosis. *Diabetes.* 1985; 34(4): 326-332. doi: [10.2337/diab.34.4.326](https://doi.org/10.2337/diab.34.4.326)

14. Heitmann RN, Sensenig SC, Reynolds CK, Fernandez JM, Dawes DJ. Changes in energy metabolite and regulatory hormone concentrations and net fluxes across splanchnic and peripheral tissues in fed and progressively fasted ewes. *J Nutr.* 1986; 116(12): 2516-2524. Web site. <http://europepmc.org/abstract/med/3543261>. Accessed December 29, 2016.

15. Heitmann RN, Dawes DJ, Sensenig SC. Hepatic ketogenesis and peripheral ketone body utilization in the nutrient. *J Nutr.* 1987; 117(6): 1174-1180. Web site. <http://europepmc.org/abstract/med/3298582>. Accessed December 29, 2016.

16. Keller U, Gerber PP, Stauffacher W. Fatty acid-independent inhibition of hepatic ketone body production by insulin in humans. *Am J Physiol.* 1988; 254(6 Pt 1): E694-E699. Web site. <http://ajpendo.physiology.org/content/254/6/E694.short>. Accessed December 29, 2016.

17. Keller U, Lustenberger M, Müller-Brand J, Gerber PP, Stauffacher W. Human ketone body production and utilization studied using tracer techniques: Regulation by free fatty acids, insulin, catecholamines, and thyroid hormones. *Diabetes Metab Rev.* 1989; 5(3): 285-298. doi: [10.1002/dmr.5610050306](https://doi.org/10.1002/dmr.5610050306)

18. Beylot M. Regulation of in vivo ketogenesis: Role of free fatty acids and control by epinephrine, thyroid hormones, insulin and glucagon. *Diabetes Metab.* 1996; 22(5): 299-304. Web site. <http://europepmc.org/abstract/med/8896990>. Accessed December 29, 2016.

19. Wiggam MI, O'Kane MJ, Harper R, et al. Treatment of diabetic ketoacidosis using normalization of blood 3-hydroxybutyrate concentration as the endpoint of emergency management. *Diabetes Care.* 1997; 20(9): 1348-1352. doi: [10.2337/diacare.20.9.1347](https://doi.org/10.2337/diacare.20.9.1347)

20. Reichard GA Jr, Skutches CL, Hoeldtke RD, Owen OE. Acetone metabolism in humans during diabetic ketoacidosis. *Diabetes.* 1986; 35(6): 668-674. doi: [10.2337/diab.35.6.668](https://doi.org/10.2337/diab.35.6.668)

21. Veech RL. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: Ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot Essent Fatty Acids.* 2004; 70(3): 309-319. doi: [10.1016/j.plefa.2003.09.007](https://doi.org/10.1016/j.plefa.2003.09.007)

22. Newman JC, Verdin E.  $\beta$ -hydroxybutyrate: Much more than a metabolite. *Diabetes Res Clin Prac.* 2014; 106(2): 173-181. doi: [10.1016/j.diabres.2014.08.009](https://doi.org/10.1016/j.diabres.2014.08.009)

23. Cox PJ, Kirk T, Ashmore T, et al. Nutritional ketosis alters fuel preference and thereby endurance performance in athlete. *Cell Metabolism.* 2016; 24(2): 1-13. doi: [10.1016/j.cmet.2016.07.010](https://doi.org/10.1016/j.cmet.2016.07.010)

24. Watanabe S, Hirakawa A, Aoe S, Fukuda K, Muneta T. Basic

- ketone engine and booster glucose engine for energy production. *Diabetes Res Open J*. 2016; 2(1): 14-23. doi: [10.17140/DROJ-2-125](https://doi.org/10.17140/DROJ-2-125)
25. Muneta T, Kawaguchi E, Nagai Y, et al. Ketone body elevation in placenta, umbilical cord, newborn and mother in normal delivery. *Glycative Stress Res*. 2016; 3(3): 133-140. Web site. <http://www.toukastress.jp/webj/article/2016/GS16-10.pdf>. Accessed December 29, 2016.
26. Utada I, Moriyama S, Hitomi S, et al. Wellness fasting for healthy longevity. *Adv Food Technol Nutr Sci Open J*. 2016; 2(3):103-109. doi: [10.17140/AFTNSOJ-2-136](https://doi.org/10.17140/AFTNSOJ-2-136)
27. Sakamoto Y, Nishizawa M, Sato T, Ohno M, Ikeda Y. Availability of body composition instrument by bio-impedance method. *Proc Japan Obesity Society*. 1992; 12: 244-246.
28. Ubukata E. Diurnal variation of blood ketone bodies in insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus patients: The relationship to serum C-peptide immunoreactivity and free insulin. *Ann Nutr Metab*. 1990; 34(6): 333-342. doi: [10.1159/000177607](https://doi.org/10.1159/000177607)
29. Watanabe S, Noboru M, Yasunari M, Ideura T. A cross-sectional study on the effects of long term very low protein diets in patients with Chronic Kidney Disease: Serum and urine DEXA and amino acid profiles. *Anti Aging Med*. 2010; 7(2): 7-13. doi: [10.3793/jaam.7.7](https://doi.org/10.3793/jaam.7.7)
30. Society of Nephrology. Web site. <http://www.jsn.or.jp/guideline/guideline.php>. Accessed December 29, 2016.
31. *Advanced Mortar System (AMOS)*. AMOS Version 22. New York, USA: IBM Inc., 2013.
32. Osame M. Hormonal change during 3 day starvation with/without glucose loading test. *Clin Funct Nutriol*. 2010; 2: 331-335.
33. Takahira H. Study on Fasting, Iwanami Book Pub, Tokyo, 1999.
34. Hirakawa A, Watanabe S, Tanaka H. Koda's fasting therapy: Energy balance and intestinal bacteria flora. *Adv Food Technol Nutr Sci Open J*. 2015; 1(5): 112-123. doi: [10.17140/AFTNSOJ-1-120](https://doi.org/10.17140/AFTNSOJ-1-120)